PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP2004/001024	02.02.2004	01.08.2003

International Patent Classification (IPC) or both national classification and IPC
A61F2/00, A61L27/38, C12N5/06, A61K31/715

Applicant
CARDIO INCORPORATED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 24

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 24 are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3,4,7
	No:	Claims	1,2,5,6,8-26
Inventive step (IS)	Yes:	Claims	7
	No:	Claims	1-6,8-26
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 24 relate to an extremely large number of possible temperature-responsive macromolecules. In fact, the claim contain so many options that a lack of clarity (and conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful examination of the claims impossible. Consequently, the examination has been carried out for those parts of the application which do appear to be clear (and concise), namely the polymers mentioned in the description at pages 62, lines 15-33 and page 76, line 20-page 79, line 4 (acrylamide derivatives (alklyl substituted) or vinylether derivatives, and those given in the examples : poly(n-polyacrylamide) or PIPAAm.

The wording of parts a) and b) from this claim ('the cell... cultured on a cell culture support grafted with a temperature responsive macromolecule having... a critical solution temperature to water') renders the matter for which protection is sought vague and unclear.

The objection could be overcome by rewording the claim to the method of detachment described in page 76, lines 20-26, page 107, last line to page 108, line 25 and page 109, line 25 to page 110, line 5. The temperature responsive macromolecule should be limited to the polymers described in pages 62, line 15-33 or page 78, line 21 to page 79, line 6.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents are referred to in this communication:

D1 : US 6 432 711 B1 (DINSMORE JONATHAN H ET AL) 13 August 2002 (2002-08-13)

D2 : WO 01/07568 A (DIACRIN INC) 1 February 2001 (2001-02-01)

D3 : KELLAR ROBERT S ET AL: "Scaffold-based three-dimensional human fibroblast culture provides a structural matrix that supports angiogenesis in infarcted heart tissue" CIRCULATION, vol. 104, no. 17, 23 October 2001 (2001-10-23), pages 2063-2068, XP002285779 ISSN: 0009-7322

D4 : SHIMIZU TATSUYA ET AL: "Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces." CIRCULATION RESEARCH. 22 FEB 2002, vol. 90, no. 3, 22 February 2002 (2002-02-22), page e40, XP002285777 ISSN: 1524-4571

D5 : TERUO OKANO: "Cardiac tissue reconstruction based on cell sheet engineering" JAPANESE CIRCULATION SOCIETY, [Online] 2002, pages 1-4, XP002285778 Retrieved from the Internet: URL:http://www.j-circ.or.jp/english/sessio ns/reports/66th-ss/okano.htm> [retrieved on 2004-06-23]

2. Article 33(2) PCT

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1-6, 10,11,16,17,22,23 is not new in the sense of Article 33(2) PCT.

2.1. Document D1 discloses (see abstract, column 5, line 62 to column 6, line 25 and examples 1-3): an embryonic stem cell which may be induced to differentiate into a desired primary cell line, such as a muscle cell line, and is cultured under conditions which provide for a three-dimensional network of contracting muscle cells which could be used to treat cardiomyopathies, myocardial infarction, congestive heart failure, or muscular dystrophy.

2.2. Document D2 discloses (see page 2, lines 18-24, page 3, lines 15-24, page 33, line 14-7): a method to prepare transplantable skeletal myoblast cells and fibroblast cells cultured on a surface coated with poly-L-lysine and laminin for treating a condition in a subject characterized by damage of cardiac tissue.

2.3. Document D3 discloses (see abstract, page 2064, left-hand column,

paragraph 2 to page 2065, right hand-column, paragraph 1): an scaffold-based three-dimensional human dermal fibroblast culture to implant angiogenic patches onto the epicardium over areas of infracted cardiac tissue. The patch contains viable cells that secrete growth factors which will simulate an angiogenic response to the damaged tissue.

3. Article 33(3)PCT.

3.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject matter of claims 3,4,12-15,18-21, 24-26 does not involve an inventive step in the sense of Article 33(3)PCT.

3.1.1. D1, which is considered to represent the most relevant state of the art to the subject matter of the present application, discloses (see above under 2.1): an embryonic stem cell which may be induced to differentiate into a desired primary cell line under specific culture conditions or by transfection with a DNA which encodes a protein or polypeptide which promotes differentiation of the stem cell into a specific cell line. The cells are cultured to from a three-dimensional structure.

3.1.2. The subject-matter of independent claim 18-21 and 24-26 differs from the disclosure of D1 in that : cells are grown on a temperature-responsive polymer such as poly(N-isopropylacrylamide) from which confluent cells detach as a three-dimensional cell sheet by reducing the temperature (from 37℃ to 20℃) without any enzymatic treatment.

3.1.3. The problem to be solved by the present invention may therefore be regarded as the provision of a three-dimensional structure comprising a monolayer or multilayer cell sheet, applicable to the heart, comprising a cell derived from a part other than myocardium of an adult.

3.1.4. In view of D4 the solution proposed in claims 18-21 and 24-26 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D4 (see abstract, page 1, left-hand column, last paragraph to right-hand column, 3rd paragraph, page 3, right-hand column, last paragraph to page 4, left-hand column, 2nd paragraph) provides a method to obtain cell sheets from neonatal rat ventricular myocytes. The cells were cultured to confluency in a 37°C incubator on temperature responsive poly(N-isopropylacrylamide) (PIPAAm) coated Petri dishes. To release confluent cells as a cell sheets, culture dishes were subsequently incubated at 20°C and the detached sheets were collected and relayered to construct pulsatile grafts in vitro.

D5 (see page 1, 3rd paragraph to page 3, 1st paragraph) describes the obtention of cell sheet engineering by appliying the temperature responsive polymer PIPAAm and the succesful establishement of single cell sheets derived from skin, retina and corneal cells, and the possibility of producing homotypically layered multiple sheets of cardiac muscle (see D4) and heterotypically layered sheets of liver, kidney and lung.

3.2. Therefore the features disclosed in D1, D4 and D5 would be combined by the skilled person, without exercise of any inventive skills in order to solve the problem posed. The proposed solution in independent claims 1,22 and 24 thus cannot be considered inventive (Article 33(3) PCT).